Supporting Information

High Resolution Parallel Reaction Monitoring with Electron Transfer Dissociation for Middle-Down Proteomics

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CONTENT:

EXPERIMENTAL SECTION

Cell Culture and Treatment

MEL cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), 1% penicillin/streptomycin, 2.5% HEPES (1M), 1% Sodium Pyruvate (100mM) and 1% L-glutamine (100X). These cells were maintained at 37°C and 5% CO_2 in suspension at a density of $1x10^5$ and $1x10^6$ cells/ml. Cell cultures were split 1:10 every two days to maintain this concentration.

To induce erythroid differentiation, cells were treated with 2% DMSO. Differentiation of these cells by DMSO is known to induce adult hemoglobin production. The untreated cells were used as control.

In order to study the time effect of DMSO induction on adult hemoglobin production the cells were treated with DMSO for 96 hours (4 days). After 4 days the cells were harvested and washed with PBS containing 100mM sodium butyrate three times. The clean cell pellets were then flash-frozen in liquid nitrogen and stored at -80°C until they were used for analysis. Treatment with DMSO slows the cell growth so that cells do not need splitting after 2 days. The cells were boosted with fresh media containing 2% DMSO at day 2 of induction. Untreated cells required splitting at day 2 to maintain the cell growth.

Sample Preparation

The frozen MEL cells were first thawed and resuspended in 1 mL hypotonic lysis buffer. Hypotonic lysis buffer leads to the swelling of cells. Histones from these cells were acid extracted with 0.4 N H_2SO_4 and precipitated with 27% (v/v) trichloroacetic acid as previously described[1]. The histone pellet was dried, resuspended in water and protein concentration was determined by Nanodrop Spectrophotometer to have an estimate of the amount of extracted histones.

HPLC Fractionation of Histones

Intact histones were fractionated using an Agilent® 1100 Series LC/MSD mass spectrometer with a 4.6 x 150 mm reversed-phase C3 column with a particle size of 5μm and a pore size of 300Å. Buffer A consisted of 94.95% water, 5% ACN, and 0.05% TFA. Buffer B consisted of 94.95% ACN, 5% water and 0.05% TFA. Approximately 200-300 μg of extracted histone pellet was dissolved in 100 μL of 0.2% formic acid. The analysis duration was 54 minutes using the following gradient: The gradient was 0-30% B in 3 minutes, 30-38% B in 30 minutes and 38-60% B in 8 minutes, 60-100% B in 3 minutes and 100% B for 10 minutes. The flow rate was 1 ml/min and it was split right before entering into the mass spectrometer. Ca. 90% of the flow was collected and 10% was directed to the MSD for fraction identification. All fractions were then dried down by lyophilization and stored at -20°C until further use.

GluC digestion

The Histone 3 fractions were selected and digested with Glu-C (Thermo Scientific) at an enzyme to protein ratio of 1:10 in 100 mM NH_4HCO_3 (pH = 4.0) at 25 °C for 5 hours. All reactions were quenched by freezing at -80 °C.

RESULTS SECTION

Figure S1: Alignment of the Histone H3 N-termini

SP|ENTRY|NAME	SEQUENCE	Monoisotopic Mass
SP|A1L0U3|A1L0U3_MOUSE	--TKQTARKSTGGKAPRKQLATKAARKSAPATGGVKKPHRYRPGTVALRE	5114.937
SP|A1L0V4|A1L0V4_MOUSE	**ARTKQTARKSTGGKAPRKQLATKAARKSAPATGGVKKPHRYRPGTVALRE**	**5338.067**
SP|P68433|H31_MOUSE	**ARTKQTARKSTGGKAPRKQLATKAARKSAPATGGVKKPHRYRPGTVALRE**	**5338.067**
SP|P84228|H32_MOUSE	**ARTKQTARKSTGGKAPRKQLATKAARKSAPATGGVKKPHRYRPGTVALRE**	**5338.067**
SP|P02301|H3C_MOUSE	ALTKQTARKSTGGKAPRKQLATKATRKSAPSTGGVKKPHRYRPGTVALRE	5341.056
SP|F8WI35|F8WI35_MOUSE	ARTKQTARKSTGGKAPRKQLATKAARKSAPSTGGVKKPHRYRPGTVALRE	5354.062
SP|P84244|H33_MOUSE	ARTKQTARKSTGGKAPRKQLATKAARKSAPSTGGVKKPHRYRPGTVALRE	5354.062
TR|E0CYN1|E0CYN1_MOUSE	ARTKQTARKSTGGKAPRKQLATKAARKSAPSTGGVKKPHRYRPGTVALRE	5354.062
TR|E0CYR7|E0CYR7_MOUSE	ARTKQTARKSTGGKAPRKQLATKAARKSAPSTGGVKKPHRYRPGTVALRE	5354.062

The N-termini of H3 (A1L0V4), H3.1 and H3.2 are identical with a monoisotopic mass of 5338.067. Histone H3 from gene Hist1h3e (A1L0U3) is a truncated version of H3, resulting in a mass of 5114.937. Histone 3.3 and H3F3a (F8WI35) is a Ala to Ser variant, resulting in a monoisotopic mass of 5354.062. Automated TREMBL entries E0CYN1 and E0CYR7 seem to be truncated versions of H3.3. We did not identify representatives of Histone H3.3 in our dataset. Histone H3C is a Arg to Leu and Ala to Thr variant, resulting in a monoisotopic mass of 5341.056. We did not identify representatives of Histone H3.C in our dataset.

Therefore, our analysis was focused on H3, H3.1 and H3.2. Since their N-termini are identical, we refer to these as H3 variants.

REFERENCES

(1) Shechter, D.; Dormann, H. L.; Allis, C. D.; Hake, S. B. *Nature Protoc.* **2007**, *2*, 1445-1457.